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RESEARCH ARTICLE

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RELATIONSHIP BETWEEN CHLOROPHYLL DENSITY AND SPAD CHLOROPHYLL METER READING FOR JERUSALEM ARTICHOKE (*Helianthus tuberosus* L.)

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SUMMARY

Chlorophyll is an indicator of crop health and productivity. Measuring chlorophyll is usually done directly and requires significant time and resources. Indirect measurement of chlorophyll density using a handheld portable chlorophyll meter can reduce time. However, this information is very limited for Jerusalem artichoke. The objectives of this study were to examine the stability of chlorophyll density and SPAD chlorophyll meter reading (SCMR) and to evaluate the relationships between chlorophyll density and SCMR for different plant genotypes, at different plant ages and planting dates. Three Jerusalem artichoke varieties were evaluated for chlorophyll density and SCMR in a greenhouse at 13 planting dates from September to March in 2008/09 and repeated in 2009/10. The treatments were replicated four times. The chlorophyll density and SCMR evaluation were carried out at 30, 60 and 90 days after transplanting (DAT). Differences among planting dates were observed for chlorophyll density and SCMR. Evaluation at 30 DAT could best discriminate the differences in chlorophyll density and SCMR among Jerusalem artichoke genotypes. High and consistent association between chlorophyll density and SCMR was found across planting dates. SCMR can be used as a surrogate trait for chlorophyll density to screen a large number of accessions in Jerusalem artichoke breeding program for high levels of chlorophyll density.

Key words: chlorophyll density, planting date, plant breeding, sunchoke

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INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus* L.) is a tuber crop, originating in North America (Kay and Nottingham, 2007). It is a versatile crop with multiple utilities and is considered to be a good source of inulin (Saengthongpinit and Sajjaanantakul, 2005). Jerusalem artichoke tubers are used as a raw material for production of health food (Chekroun *et al*., 1996; Kaur and Gupta, 2002; Orafti, 2005; Gaafar *et al*., 2010), animal feed (Seiler and Campbell, 2004) and bioethanol, and consumed as fresh vegetable (Denoroy, 1996).

Chlorophyll is a major photosynthetic pigment. The major roles of this pigment are to absorb and react with visible light in photosynthesis (Taiz and Zeiger, 2010). Chlorophyll content per unit leaf area (chlorophyll density) has been used as an index of photosynthetic capacity and growth of many crop plants and it is most important crop growth parameter to determine the performance of crop growth (Bowyer and Leegood, 1997). High biomass production of Jerusalem artichoke is at least partly due to its above-average photosynthetic rate (Soja and Haunold, 1991) and photosynthetic activity (Sawicka and Michalek, 2005). Photosynthetic rates of cultivars are positively correlated with chlorophyll concentrations in the leaves. For improving Jerusalem artichoke one can take advantage of superior photosynthetic capacity at the leaf, plant and canopy level, but screening for photosynthetic characteristics has been proven to be successful only when the sink capacity of the tubers is also improved (Soja and Haunold, 1991).

Several complex laboratory methods are available for assessing chlorophyll density. Chlorophyll is not soluble in water but it can be extracted in 80% (v/v) acetone solution (Arnon, 1949) or N, N-dimethylformamide (DMF) (Moran, 1981) and then its content can be determined by using a spectrophotometer. This measurement process is laborious, time consuming, costly, destructive and inconvenient for large number of samples to be analyzed in a short period of time. Indirect methods to assess chlorophyll density which are easy and rapid to use, economical, effective and reliable to make chlorophyll density measurements are needed.

Minolta SPAD-502 meter (Tokyo, Japan) has been developed to assess crop health and has been widely used by scientists conducting experiments in different areas of plant sciences (Markwell *et al*., 1995). The SPAD (soil plant analysis development) meter measure green color intensity in leaves in *vivo*, and is an ideal instrument for collecting large amount of data on chlorophyll in the field within a short time without any destructive sampling. Close associations between SCMR value and chlorophyll density have been reported for maize and soybean (Markwell *et al*., 1995), cotton (Wu *et al*., 1998), rice (Jinwen *et al.*, 2009), potato (Bindi *et al*., 2002), wheat (Ommen *et al*., 1999; Udding *et al*. 2007), lauraceae, lindera, pondberry (Hawkins *et al*., 2009) and peanut (Arunyanark *et al*. 2009).

However, lack of good correlation between SCMR and chlorophyll density was reported for Amur Maple, when it was evaluated for tolerance to lime-induced iron chlorosis (Barwinsky and Remphrey, 2009). Previous studies have also indicated association and non association between SCMR and chlorophyll density depends on crops and genotypes. In Jerusalem artichoke, variation in SCMR was observed among different clones (Serieys *et al*., 2010). However, to the best of our knowledge during the literature review, no study has been conducted to develop relationships between SCMR and chlorophyll density for Jerusalem artichoke. The use of SCMR to assess relative chlorophyll density as alternative to the standard method is very attractive because it is easy to operate, low cost, non-destructive and can be applied in the field conventionally. SCMR could help to increase the effectiveness of breeding if it can be used to identify Jerusalem artichoke genotypes with high chlorophyll density and high biomass productivity.

Therefore, the objectives of this study were to examine the suitability of chlorophyll density and evaluate the relationships between chlorophyll density and SCMR for different plant genotypes, plant ages, and planting dates. The results will help clarify the efficacy and efficiency of the technique and its potential usefulness in breeding Jerusalem artichoke.

MATERIALS AND METHODS

Plant material

Three Jerusalem artichoke varieties (CN 52867, JA 89 and HEL 65) were used in this study. The CN 52867 is an early maturing variety, JA 89 is intermediate maturity, and HEL 65 is late maturing variety. They also differ in leaf color. They were grown in pots assigned in a complete randomized design with four replications and 13 planting dates for two years during the months of September 2008 to March 2009 and September 2009 to March 2010 at the Field Crop Research Station, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand (latitude 16 °26´N, longitude 102° 48´E).

Each plant was grown in a plastic pot 28 cm in height and 31 cm in diameter, and there were 3 plants in a replication for each treatment. Therefore, there were 36 plants (pots) totally for each planting date. A series of 13 planting dates were evaluated with 15 days intervals, starting from 20th September to 20th March for both years. These planting dates were selected because they are representative of the planting dates which coincides with the short photoperiod time of the year.

The pot contained 23 kg of soil and burnt rice husk at the ratio of 1:1 by volume. The particles of mixed soil when analyzed resulted in 69% sand, 19% silt and 12% clay for first year and 80% sand, 16% silt and 4% clay for the second year.

Uniform seed tubers were used as planting materials. They

were cut into small tuber pieces with two to three buds. These tuber pieces were then pre-sprouted in coconut peat medium under ambient conditions for 4-7 days and later they were transferred to germinating plug trays with mixed medium containing burnt rice husk and soil for 7 days for complete sprouting. Well-sprouted and uniform tuber pieces were selected for planting.

Weeds were manually controlled throughout the experiment and the combination of N-P_2O_5-K_2O fertilizer (15-15-15) at the rate of 2 g per pot at 30 DAT. All pots received equal amounts of water daily to avoid any stress.

Data collection

Data were collected using the SCMR and chlorophyll content at 30, 60 and 90 DAT for each planting date. The data was later converted into chlorophyll density (chlorophyll content per unit leaf area).

A plant from each plot was randomly sampled, and SCMR was recorded from the third fully-expanded and intact leaf from the top of the main stem between 09:00am–11:00am hours. A Minolta SPAD-502 meter was used in recording SCMR on either side of the midrib and the data were averaged as a single value for each leaf.

The intact leaf previously used for recoding SCMR was further used for determining chlorophyll content. The leaf was bored by a disc borer with 1 cm^2 in leaf area and a leaf disc was soaked in 5 ml of *N (N*-dimethylformamide) and kept in the dark for 24 hrs before determining chlorophyll using a light absorption technique with a spectrophotometer. Chlorophyll Density (per unit leaf area) was determined following the procedures described by Moran (1981).

Statistical analysis

Analysis of variance was performed for individual seasons, error variances were tested for homogeneity and combined analysis of variance for each variable was performed across years and planting dates (Gomez and Gomez, 1984). There were significant G x E interactions for all characters, and data of individual seasons were reported. Least significant difference (LSD) was used to compare means. Simple correlations among and within main effects (year, planting date and genotype) were computed for SCMR and chlorophyll density. The analysis was done using statistix8.

RESULTS

Analysis of variance

Differences between years were statistically significant for chlorophyll density at 30 and 90 DAT but not for 60 DAT. Differences between years were also statistically significant for SCMR at 30 and 90 DAT (Table 1). Differences in planting dates were also statistically significant for chlorophyll density and SCMR at 30, 60 and 90 DAT. Differences among Jerusalem artichoke genotypes were statistically significant for chlorophyll density and SCMR at 30, 60 and 90 DAT.

The interactions between year and planting date were significant for chlorophyll density and SCMR at 30, 60 and 90 DAT. The mean squares for these interactions were rather similar to those of planting date main effects. The interactions between genotype and year and the interactions between genotype and planting date were significant for chlorophyll density and SCMR at most sampling dates except for chlorophyll density at 60 DAT. The mean squares of the interactions were generally lower than those of planting date main effect and genotype main effect. The secondary interactions (Y x PD x G) were significant for chlorophyll density and SCMR at 30, 60 and 90 DAT. The mean squares for these interactions were generally smaller than those of genotype main effect.

Chlorophyll density

Chlorophyll densities at 30 DAT across all planting dates and years ranged from 6.3 to 13.4 µg/ cm^2 (Figure 1). Chlorophyll densities in general were higher at 60 DAT and lowest at 90 DAT. Among the varieties tested CN 52867 was generally higher than JA 89 and HEL 65 for chlorophyll density at 30 DAT and 60 DAT across all planting dates for two years. Genotype x planting date interactions were rather low at 30 DAT (Table 1). The results showed that chlorophyll density at 30 DAT could be able to illustrate differences among Jerusalem artichoke accessions.

The interactions between genotype and planting date were higher at 60 DAT, and, therefore, separation of the Jerusalem artichoke genotypes using chlorophyll density was difficult at this stage. High interactions between genotype and planting date were observed for chlorophyll density evaluated at 90 DAT (Figure 1). The identification of the best accession was most difficult because the performance of Jerusalem artichoke accessions for chlorophyll density was not consistent across planting dates and years.

SPAD chlorophyll meter reading

SCMR at 30 DAT across planting dates and years ranged from 23.9 to 47.2 and followed similar patterns as observed for chlorophyll content (Figure 2). Means for SCMR in 2008/09 were 36.0 at 30 DAT, 33.2 at 60 DAT and 38.7 at 90 DAT, whereas, means for SCMR in 2009/10 were 39.4 at 30 DAT, 27.2 at 60 DAT and 30.5 at 90 DAT. The accession CN 52867 in general had the highest SCMR at 30 DAT across planting dates for both years, whereas the accessions JA 89 and HEL 65 performed very similar and the interactions between genotypes and planting dates seemed to be limited between the accessions JA 89 and HEL 65. The accession CN 52867 was clearly separated from the accessions JA 89 and HEL 65 especially in the year 2008/09.

Table 1. Mean square of combined analysis of variance for chlorophyll density (µg/cm^2) and SPAD chlorophyll meter readings (SCMR) of three genotypes for thirteen planting dates during the two year study period (2008/09 and 2009/10).

Source of variation	df	Chlorophyll density (µg/cm^2)			SCMR		
		30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
Year (Y)	1	99.0**	1.0 ns	18.2**	626.7**	35.1 ns	939.5**
Planting Date (PD)	12	15.6**	61.2**	45.5**	169.5**	199.7**	363.0**
Y x PD	12	15.6**	58.2**	33.1**	107.4**	286.3**	496.6**
Genotype (G)	2	129.7**	107.3**	135.4**	158.4**	166.4**	972.8**
Y x G	2	5.3**	1.5 ns	18.2**	45.9**	23.2*	332.6**
PD x G	24	2.8**	11.2**	10.0**	15.8**	54.3**	83.4**
Y x PD x G	24	2.1*	16.1**	16.2**	11.4**	50.4**	173.5**
Pooled error	228	0.9	1.6	1.2	4.0	6.3	10.5

ns, *, ** Non significant, significant at $P \leq 0.05$ and $P \leq 0.01$ respectively.
DAT = Day after transplanting

Table 2. Simple correlation coefficients between chlorophyll density (µg/cm^2) and SPAD chlorophyll meter reading (SCMR) of three genotypes for thirteen planting dates during the two year study period (2008/09 and 2009/10).

Varieties	2008/09			2009/10		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
CN 52867	0.84**	0.95**	0.94**	0.93**	0.89**	0.84**
JA 89	0.92**	0.96**	0.90**	0.77**	0.79**	0.89**
HEL 65	0.81**	0.92**	0.90**	0.90**	0.87**	0.93**

** Significant at $P \leq 0.01$
DAT = Day after transplanting

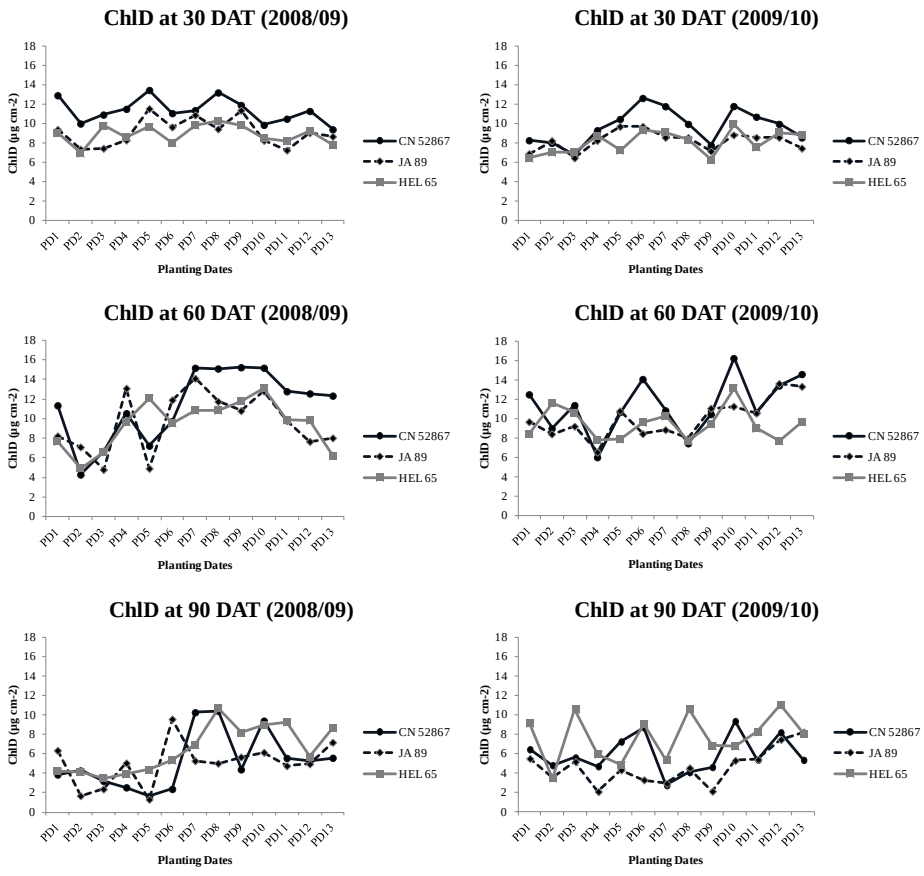

Figure 1. Chlorophyll density (ChlD) at 30, 60 and 90 days after transplanting (DAT) of three Jerusalem artichoke varieties evaluated for thirteen planting dates (PD) during the two year study period (2008/09 and 2009/10).

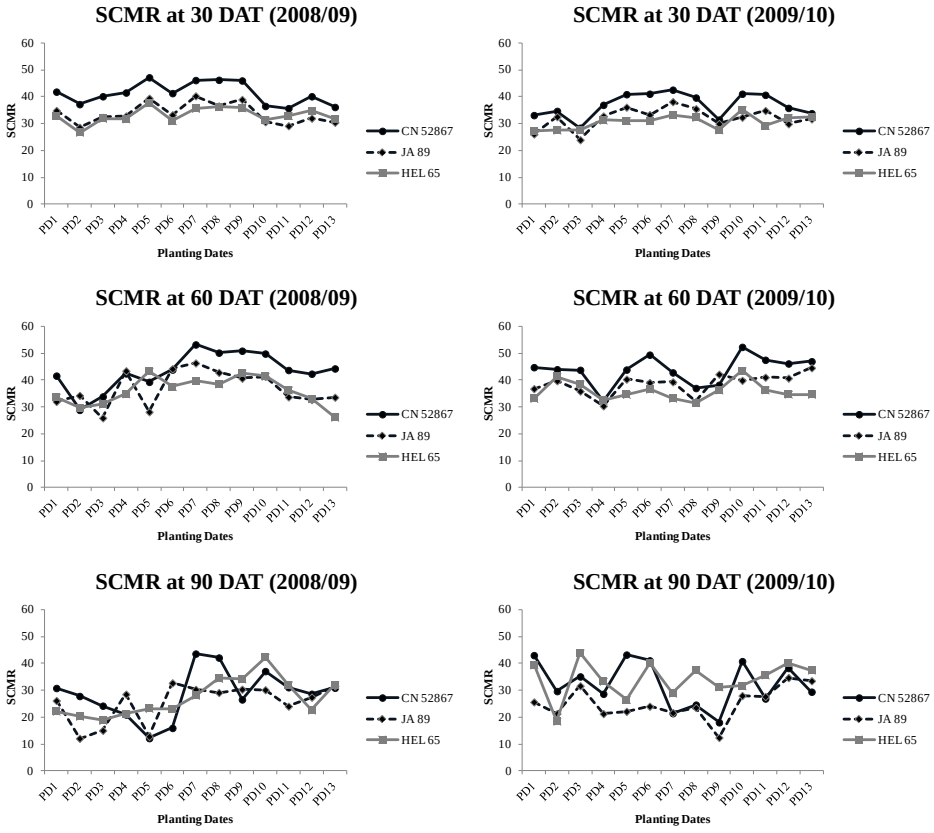

Figure 2. SPAD chlorophyll meter reading (SCMR) at 30, 60 and 90 days after transplanting (DAT) of three Jerusalem artichoke varieties evaluated for thirteen planting dates (PD) during the two year study period (2008/09 and 2009/10).

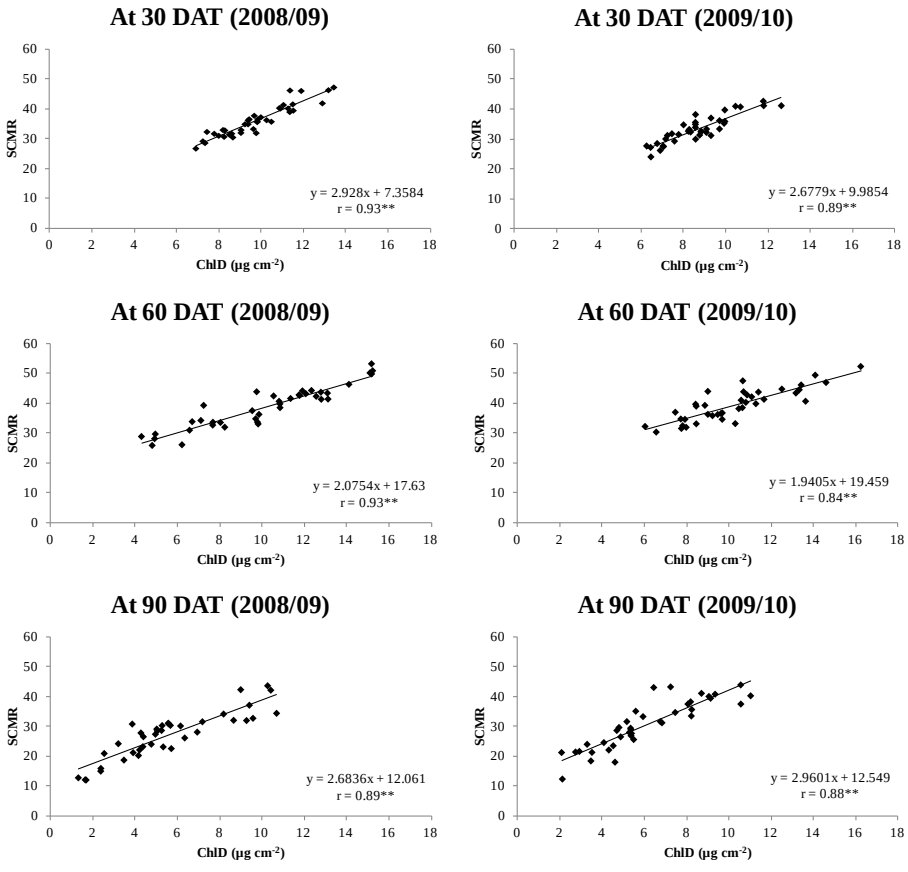

Figure 3. Correlation between chlorophyll density (ChlD) and SPAD chlorophyll meter reading (SCMR) at 30, 60 and 90 days after transplanting (DAT) of three Jerusalem artichoke varieties evaluated for thirteen planting dates during the two year study period (2008/09 and 2009/10).

The highest SCMR were observed in the accession CN 52867 in most planting dates for both years when evaluated at 60 DAT (Figure 2). However, the differences between accessions were smaller than those at 30 DAT because of high genotype x planting date interactions.

Genotype x planting date interactions for SCMR was highest at 90 DAT and the identification of genotypes with high SCMR was difficult (Figure 2). The performance of the accessions was not consistent across planting dates or years.

Relationship between chlorophyll density and SCMR

Chlorophyll contents of three Jerusalem artichoke genotypes across 13 planting dates for two years at 30, 60 and 90 days after transplanting were plotted against SCMR (Figure 3). Only one figure is showed because all correlation coefficients followed a similar pattern. When three genotypes were combined across planting dates, the correlation coefficients were 0.93 for 30 DAT, 0.93 for 60 DAT and 0.89 for 90 DAT in the year 2008/09. Similarly, the correlation coefficients were 0.89 for 30 DAT, 0.84 for 60 DAT and 0.88 for 90 DAT in the year 2009/10.

Positive and significant correlations coefficients were observed between chlorophyll contents and SCMR for all genotypes (Table 2). The CN 52867 had the correlation coefficients ranging between 0.84 and 0.95, and the correlations coefficients were similar between two years. The JA 89 genotype had correlation coefficients ranging between 0.77 and 0.96 and the correlation coefficients in the year 2008/09 were higher than those in the year 2009/10. The HEL 65 variety had correlation coefficients between 0.85 and 0.93 and the correlation coefficients were rather similar during both the years. High and positive correlations between the traits indicated good relationship and the consistency of the results across planting dates and years indicated accuracy of the method used.

DISCUSSION

Determination of chlorophyll density is costly, laborious, destructive and time consuming. This method, although most reliable, is not suitable for use in screening of a large number of genotypes for evaluating chlorophyll content and in the evaluation of plant health status. Other methods that are not expensive, non-destructive and user friendly are worth exploring. In this study, chlorophyll density and SCMR were compared for a range of planting dates and Jerusalem artichoke genotypes for two years. The key objective was to find out if SCMR can be used as an alternative method for direct evaluation of chlorophyll density. To be useable, SCMR readings should be highly correlated with chlorophyll density across planting dates and genotypes that were evaluated for Jerusalem artichoke.

Differences between years for chlorophyll density and SCMR were significant for most evaluation times at 30, 60 and 90

DAT. This could be due to differences in environmental conditions as the greenhouse was not fully controlled. Temperature would be the most possible cause for this variability because it was highly variable in the greenhouse during this study.

Differences among planting dates for chlorophyll density and SCMR could be largely due to the differences in temperature and day length. Day length and temperature are possibly the main causes of the variability on chlorophyll density. In addition, Puangbut *et al.* (2011) reported that the temperature was influenced on inulin content and inulin yield. Temperature is highly variable during this growing period from September to March, whereas the day length is longer (12.00 – 12.33 hr) in September and March and shorter (11.20 hr) in December.

Genotypes were also significantly different for chlorophyll density and SCMR and the differences were more pronounced at 30 DAT. The results indicated that chlorophyll density and SCMR are equally effective in discriminating the genotypic differences and the 30 DAT was the best time for making evaluations for chlorophyll contents. This could be due to the change in chlorophyll content in leaves at later evaluation times that might have caused large variations by the translocation of nutrients from leaves to tubers of Jerusalem artichoke. The greatest reduction in SCMR and chlorophyll density was observed at 90 DAT.

Among 169 Jerusalem artichoke accessions, Serieys *et al.* (2010) found high variation in SCMR and the selection of Jerusalem artichoke for high SCMR is possible. The SCMR was also different, depending on plant ages. Soja and Haunold (1991) found that chlorophyll concentration in Jerusalem artichoke reduced during flowering and the tuber loading growth stages. Thus, tuber yield of Jerusalem artichoke depends mainly on assimilates from lesser part of the leaves, and on stored assimilates from the stem, to fill the tuber.

Chlorophyll density and SCMR were consistent across planting dates showing the repeatability of the methods especially at 30 DAT. The results were less consistent at 60 and 90 DAT due to high genotypes and planting date interactions. The good relationship between chlorophyll density and SCMR across planting dates indicated the possibility to use SCMR as a surrogate trait for chlorophyll density in Jerusalem artichoke. SPAD-502 chlorophyll meter could be used for indirectly assessing chlorophyll density in Jerusalem artichoke for a range of growing conditions evaluated in this study. The method should be applicable to screening of a large number of Jerusalem artichoke genotypes for high chlorophyll contents.

The differences in the results of different evaluation times were due to the nature of the traits rather than the precision of the methods. The high and consistent correlation coefficients between chlorophyll content and SCMR across planting dates and genotypes supported these

observations and key conclusions of this study.

CONCLUSION

This study resulted in following key conclusions:

1. The results from two year studies that were conducted under greenhouse environmental conditions showed good relationships between chlorophyll density and SCMR across Jerusalem artichoke genotypes and planting dates.
2. Among the two methods that were used to determine chlorophyll contents were good and reliable in detecting the variations in Jerusalem artichoke. However, SCMR is more user-friendly, less expensive and non-destructive and it can be used as an alternative method for chlorophyll density at any planting date in the range of planting dates studies.
3. If one has to screen a large number of accessions in Jerusalem artichoke breeding program for higher levels of chlorophyll density, evaluation at 30 DAT is recommended. Evaluation later than 30 DAT could result in high genotype x planting date interaction and the identification of superior genotypes would become difficult.

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